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Project Title: Managing Watergrass (*Echinochloa* spp.) Resistance To Rice Herbicides in an Aquatic Environment: Research and Demonstration in Affected Farms

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ABSTRACT

California rice is being subjected to an epidemic of herbicide resistance in watergrass (*Echinochloa* phyllopogon and *E. oryzoides*) populations. Watergrasses are the major weeds of rice and the development of herbicide resistance not only deprives farmers of essential tools for weed control, but also results in increased herbicide rates and frequency of application. This problem has, thus, serious economical and environmental implications. The objective of the 2000 activity was to conduct the second year of a medium-term field experiment initiated under this grant in 1999 to evaluate in a systems approach key management options for reducing herbicide selection pressure towards resistance, namely: 1) annual rotation to herbicides with different mechanisms of action or tank mixtures and sequential applications of herbicides with different mechanisms of action, and 2) the use of transgenic rice cultivars resistant to environmentally friendly broad-spectrum herbicides, and 3) reducing seed survival with appropriate straw management practices by initiating a comparison of the seed survival rate under straw cover, straw removal, and straw incorporated post-harvest management practices which are expected to affect the soil re-infestation with herbicide-resistant seed. Prevention of reseeding is an essential component for arresting the development of herbicide resistance

in weed populations. This year's activities also involved tracking treatment effects on seed bank populations; using the research site as a field demonstration site by conducting a field day in collaboration with UC Cooperative Extension personnel; discussing results from two years of this project at the UCCE winter farm meetings; and making the site available for researchers interested in concurrent issues. This experiment was implemented in a conventional rice grower's field in Glenn Co. where lack of watergrass control with molinate, thiobencarb, and fenoxaprop has been repeatedly observed. In this second year of the project the herbicide trial was planted, treated and evaluated, an expanded site area was prepared for the application of straw management treatments after harvest, part of a demonstration area was utilized to test tank mixed combinations of herbicides on this resistant watergrass population, and a separate small trial was installed and conducted that explored alternative chemistries and the alternative planting methods of drill and no-till seeding. Several important results were obtained this season: 1. Higher than expected recruitment of new seed from rather low plant densities indicated the necessity of better herbicidal control to prevent reseeding. 2. The upper layers of soil were depleted of old seed significantly more than the lower levels. 3. The seed bank was reduced over winter and spring by 95% after burning and flooding straw treatments. 4. Emergence of watergrass seedlings was 30% of the preplant seed bank in 2000 contrasted with 3.5% in 1999. Tracking watergrass plant densities throughout the season provided a means for evaluating the effects of two herbicide applications in each of the four treatments. These data indicated the failure of molinate to provide acceptable control on this resistant population even with split applications of a higher total rate and with improved water management. The two applications of all three foliar treatments achieved levels of control judged probably adequate to deplete the seed bank by limiting the recruitment of new seed, and possibly intensifying selection pressure enough to alter the patterns of resistance in the seed bank. This project will be a major contribution towards establishing herbicide strategies and cultural practices for controlling resistant watergrass populations. At the same time, this project will advance the integration of population and seed bank dynamics with herbicide strategies and evolution of resistance concepts producing more comprehensive models for long term planning.

EXECUTIVE SUMMARY

Because of an epidemic of herbicide resistance in watergrass (*Echinochloa phyllopogon*, and *E. oryzoides*) in California rice, the first year of a three- to four-year study was begun for understanding the role of herbicide management in delaying the development of resistance. The experiment was conducted during the 1999 season on a cooperating farmer's rice field in Colusa Co., CA. This field is heavily infested with early watergrass (*E. oryzoides*), with resistance to thiobencarb, bispyribac-sodium and fenoxaprop-ethyl. Research focuses on developing and demonstrating knowledge on rational herbicide use strategies for resistance management, which is essential for the implementation of sustainable integrated watergrass management strategies Watergrasses are the major weeds of rice and the development of herbicide resistance not only deprives farmers of essential tools for weed control, but also results in increased herbicide rates and frequency of application. This problem has, thus, serious economical and environmental implications. The objective of the 2000 activity was to conduct the second year of a medium-term field experiment initiated under this grant in 1999 to evaluate in a systems approach key management options for reducing herbicide selection pressure towards resistance, namely: 1) annual rotation to herbicides with different mechanisms of action or tank mixtures and sequential applications of herbicides with different mechanisms of action, and 2) the use of transgenic rice cultivars resistant to environmentally friendly broad-spectrum herbicides, and 3) reducing seed survival with appropriate straw management practices by initiating a comparison of the seed survival rate under straw cover, straw removal, and straw incorporated post-harvest management practices which are expected to affect the soil re-infestation with herbicide-resistant seed. Prevention of reseeding is an essential component for arresting the development of herbicide resistance in weed populations. This year's activities also involved tracking treatment effects on seed bank populations; using the research site as a field demonstration site by conducting a field day in collaboration with UC Cooperative Extension personnel; discussing results from two years of this project at the UCCE winter farm meetings; and making the site available for researchers interested in concurrent issues. This experiment was implemented in a conventional rice grower's field in Glenn Co. where lack of watergrass control with molinate, thiobencarb, and fenoxaprop has been repeatedly observed. In this second year of the project the herbicide trial was planted, treated and evaluated, an expanded site area was prepared for the application of straw management treatments after harvest, part of a demonstration area was utilized to test tank mixed combinations of herbicides on this resistant watergrass population, and a separate small trial was installed and conducted that explored alternative chemistries and the alternative planting methods of drill and no-till seeding. Several important results were obtained this season: 1. Higher than expected recruitment of new seed from rather low plant densities indicated the necessity of better herbicidal control to prevent reseeding. 2. The upper layers of soil were depleted of old seed significantly more than the lower levels. 3. The seed bank was reduced over winter and spring by 95% after burning and flooding straw treatments. 4. Emergence of watergrass seedlings was 30% of the preplant seed bank in 2000 contrasted with 3.5% in 1999. Tracking watergrass plant densities throughout the season provided a means for evaluating the effects of two herbicide applications in each of the four

treatments. These data indicated the failure of molinate to provide acceptable control on this resistant population even with split applications of a higher total rate and with improved water management. The two applications of all three foliar treatments achieved levels of control judged probably adequate to deplete the seed bank by limiting the recruitment of new seed, and possibly intensifying selection pressure enough to alter the patterns of resistance in the seed bank. This project will be a major contribution towards establishing herbicide strategies and cultural practices for controlling resistant watergrass populations. At the same time, this project will advance the integration of population and seed bank dynamics with herbicide strategies and evolution of resistance concepts producing more comprehensive models for long term planning.

The experiment has also demonstrated its potential as a much-needed tool to enhance farmer's awareness of the magnitude of the herbicide resistant problem that is crippling weed control in California rice. At the same time, through the field tour and subsequent presentations at growers' meetings, valuable concepts and techniques from the "real world" are being forwarded to growers. The high turnout to these events are testimony of farmers' need for solutions and of the relevance and potential of this applied research and demonstration project.

REPORT

Introduction

Pest management in rice is exceedingly complicated by the flooded nature of rice culture and by the lack of rotation to other crops due to the poorly drained nature of the heavy clay rice soils. Herbicides, which are still the main tool for weed control in rice, are applied into an aquatic environment, raising concerns about water quality and aquatic organism health. Ground applications are difficult and slow on flooded fields, thus most herbicides are applied by air. Herbicide drift has often resulted in injury to neighboring crops, such as walnuts, fruit trees and cotton. Concerns about crop safety and environmental health in California have restricted the availability of herbicides for rice compared to other crops.

Continuous rice, the limited opportunities for cultural control, and the few available chemical tools have resulted in the repeated use of herbicides with the same mechanism of action for the control of watergrass (*Echinochloa phyllopogon*, and *E. oryzoides*), which are the worst weeds of California rice. The herbicides available for watergrass control in rice (propanil, molinate, thiobencarb, and fenoxaprop) represent only three different mechanisms of action. The frequent application of herbicides with the same mechanism of action has exerted significant selection pressure on watergrass populations in favor of herbicide-resistant watergrass biotypes. Herbicide resistance is not new to California rice. In fact, resistance to bensulfuron (Londax () in broadleaf weeds and sedges has reached epidemic proportions in the recent past. Most rice farmers in California cannot use this herbicide any longer; substitute herbicides have offered only partial help. In 1999 a new herbicide introduced to replace bensulfuron has resulted in severe drift injury to prune trees. Recent data also indicates that watergrass exhibits cross- and multiple resistance to existing and new, still unregistered, herbicides. In many cases watergrass accessions collected from rice fields have tested resistant to three of the four available herbicides. The exception was propanil; this has prompted for increased use of this herbicide, which requires very judicious use to prevent damage to fruit trees from spray drift. Due to proximity to fruit trees and cotton the use of this herbicide is restricted for many areas of California rice. Herbicide resistance thus severely reduces farmers' options for weed control. Weed control failure due to resistance usually leads to increased dosages and number of applications, along with complex herbicide combinations, that compromise water and environmental quality, the cost of weed control, the safety to rice and neighboring crops, and the economic viability of California's rice industry. Herbicide resistance in watergrass has reached epidemic proportions, but we are at a window where development and demonstration of resistance management strategies may have significant long-term results in delaying the development of resistance, and avoiding futile herbicide overuse.

Because of this new resistance epidemic in California rice, the University of California at Davis has undertaken a medium-term study to examine the effects of new methodologies in reducing infestations by herbicide-resistant watergrass. Since herbicide use is the driving force of this process, and herbicides are an essential tool for weed control as well as an environmental concern, it is of paramount importance that we understand the role of herbicide management in delaying the development of resistance. Scientifically validated knowledge in this area is woefully lacking. This research thus focuses on developing and demonstrating knowledge on herbicide use strategies, including the use of herbicide-resistant rice cultivars, for resistance management. This knowledge is essential to allow for the successful implementation of integrated management strategies, where herbicide use is complemented by other non-chemical weed control options.

The objective of the 2000 activity was to conduct the second year of a medium-term field experiment initiated under this grant in 1999 to evaluate in a systems approach key management options for reducing herbicide selection pressure towards resistance, namely: 1) annual rotation to herbicides with different mechanisms of action or tank mixtures and sequential applications of herbicides with different mechanisms of action, and 2) the use of transgenic rice cultivars resistant to environmentally friendly broad-spectrum herbicides, and 3) reducing seed survival with appropriate straw management practices by initiating a comparison of the seed survival rate under straw cover, straw removal, and straw incorporated post-harvest management practices which are expected to affect the soil re-infestation with herbicide-resistant seed. This year's activities also involved tracking treatment effects on seed bank populations and proportions of resistant weeds over a three-year period. The research site was also used as a field demonstration site by conducting a field day in collaboration with UC Cooperative Extension (UCCE) personnel; results from the activities conducted in 1999-2000 were presented at the UCCE winter grower meetings.

The first two years of this field research were implemented in a conventional rice grower's field in Glenn Co. near the Princeton/Norman Road area of the northern Sacramento valley where lack of watergrass control with molinate, thiobencarb, and fenoxaprop has been repeatedly observed.

Materials and Methods

Three experiments were conducted in 2000 and a fourth one was initiated:

1. **Main Experiment** consisting of large plots to test herbicide management strategies for delaying the development of herbicide resistance in watergrass.
2. An **Exploratory trial** to study the potential for no-tillage to reduce watergrass emergence.
3. A **Foliar Herbicide Screening trial** to test herbicides and combinations of herbicides for in-season control of herbicide-resistant watergrass.
4. The experiment initiated this year is a **Straw Management trial** to establish a non-chemical cultural practice to reduce the soil reservoir of watergrass seed.

Post-harvest 1999 seed bank samples. One four-inch diameter soil core was taken at each of the eight positions in each plot that were sampled preplant in 1999 (Fischer and Hill 2000). The eight cores per plot were divided into an upper 0 to 2 inch depth section and a lower 2 to 4 inch depth section. Bulking by depth and plot gave a total sampled quadrat area of 0.06 m^2 per plot. These samples were placed in screen mesh bags of 1mm opening size, washed in a conventional washing machine, and dried. Apparently viable early watergrass seed were extracted from the residue with a forceps and counted. New early watergrass seed shed in 1999, complete with intact awns and glumes covering a shiny seed coat were readily distinguished from the old seed, which had faded, or missing glumes and awns and a dull fragile seed coat. Barnyardgrass seed, present in much smaller numbers was identified by its smaller seed size and counted separately.

Experimental layout, seedbed preparation. In this second year of the project, the original 11.3 acres of the Main Experiment was expanded to accommodate the straw management trial so that the total experimental area now includes an entire check of 21.2 acres (Figure 1). This entire check received a fall burn and winter flood straw management treatment and was drained on 2/28/00. Early spring watergrass germination was assessed on 3/24/00 using a 1 square foot quadrat placed eight times in each of the 16 plots of the Main Experiment as well as in the southwest area of the Exploratory and Herbicide Screening trials and in the Straw Management area. Mr. Larry Maben, cooperating grower, rebuilt the main irrigation supply levee and prepared the seedbed with chisel, disk, and plane. The narrow plots of the Main Experiment were planed with a small implement borrowed from the Rice Experiment Station. Maintenance of old levees and the installation of new ones in the southwest demonstration area (Figure 1) was done by a local contractor. Heavy rain on 4/16 and 17 interrupted seedbed preparation and germinated a new flush of watergrass seed, which was then counted as before on 4/25. Ammonium sulfate at 126 lb N/ac was flown onto all plots, disk incorporated, and followed by another application of starter fertilizer at 32 lb N/ac which was not incorporated. The straw trial was reworked with chisel, disk, plane and roller on 4/29-5/1. We were unable however, to replane or roll within the Main Experiment plots leaving an acceptable, but less than ideal seedbed.

Auxiliary trials and demonstration area. The experimental area available was utilized to the maximum extent possible (Figure 1) by addressing two other important aspects of resistance management of interest to growers and useful in guiding our selection of treatments for the Main Experiment and Straw Management treatments: a) a small-plot Foliar Herbicide Screening trial provided information on which combinations of available and new herbicides worked best on this resistant watergrass population. It consisted of 4 replications of ten treatments, randomized complete block design, in 10 ft x 20 ft plots. Treatments were applied at the 2-3 tiller stage of rice 33 DAS with backpack sprayer. B) The Exploratory trial investigated unconventional planting methods (drill and no-till seeding) and techniques for using alternative chemistries (pendimethalin). This trial was laid out in nine plots separated by levees, with a water supply ditch and a drain ditch that allowed individual water management. Three treatments were assigned to plots in a RCB design with 3 replications. 1. The molinate treatment received the same applications and timing of Ordram as in the Main Experiment. 2. The objective of the no-till treatment in this trial was to demonstrate the potential for depleting the upper soil layer of seed by minimizing reseeding, enhancing spring germination, not turning up old buried seed, thus minimizing watergrass emergence at planting time. These plots received two applications of a 2% glyphosate solution 5 days before seeding – no herbicides were used after seeding. 3. The pendimethalin plots of this trial had dry seed raked in (to simulate drill seeding), flooded the next day, and drained the next. Heavy rains and broken levees the following day reflooded the plots. They were drained again and an application of Prowl 3.3 EC, 1 lb ai/ac in 20 GPA was made at 5 DAS. These plots were kept drained with a small pump but again received heavy rains at 8 and 9 DAS.

Preplant seed bank samples. Soil samples in the Main Experiment were taken to a depth of 15 cm from the same 8 points in each plot that were sampled the previous year. Soil was bulked, mixed, sub sampled (4,000 cm^3 per plot), washed, and seed extracted as described above. Samples were also taken from the nine plots of the exploratory trial in a slightly different manner. Four 10 cm diameter cores were taken per plot to a depth of 10 cm in a stratified random sampling pattern. Each core was divided into the top 2.5 cm and the lower 7.5 cm, bulked by plot and depth, and processed as described above.

Planting. On 5/5 dry seed (variety M202 and Liberty-Link) was applied to the Main Experiment plots with backpack air seeders at a rate of 150 lb/ac. The entire 21.2-acre experimental area was flooded on 5/6. Calcium chloride was supplied in the floodwater at a rate of 10 lb/ac. The accessory demonstration area was water seeded with presoaked seed by backpack seeder on 5/6 and 5/8. The straw trial and equipment transit areas (Figure 1) were seeded with presoaked M202 seed by airplane on 5/11. Water was maintained at a depth of 4 to 6 inches in all plots. Due to poor germination of the Liberty Link seed, the four plots of the continuous Liberty treatment had to be reseeded with 150 lb/ac of presoaked seed on 5/23, 17 days after seeding (DAS).

Herbicide applications in the Main Experiment. In order to achieve significant seed bank depletion by preventing the late emergence and reseeding noted in the first year, a strategy of two herbicide applications was adopted for all treatments. All spray applications (treatments 2, 3, and 4) were applied to 4-leaf stage rice (lsr) at 28 DAS followed by (fb) the second application at 54 DAS applied to well tillered rice. The treatments were as follows: 1. Continuous molinate: Ordram 15 G was applied by hand with a belly grinder at a rate of 3 lb ai/ac at 1 lsr 12 DAS fb 2 lb ai/ac at 2.5 lsr 20 DAS. At 54 DAS these plots also received an application of 24 oz/ac of MCPA for control of smallflower umbrellaplant (*Cyperus difformis)*. 2. Intensive Strategy: This treatment is intended to be an intensive control strategy employing tank mixes of herbicides with different modes of action. This year we used Abolish 8EC (thiobencarb) 4lb ai/ac plus Regiment 80WP (bispyribac) at 15 g ai/ac fb Clincher (cyhalofop) at 210 g ai/ha plus Superwham (propanil) 6 lb ai/ac. 3. Herbicide Mode of Action Rotations: This year the treatment was "rotated" from the glufosinate applied last year to propanil; Superwham at 4 lb ai/ac fb Superwham at 6 lb ai/ac. 4. Continuous glufosinate: Liberty 1.67SL at 350 g ai/ha fb Liberty 1.67SL at 500 g ai/ha. (We intended to apply 500 g ai/ha at the first application but 350 g ai/ha was applied by mistake.) The grower made all of the first applications with his ground rig equipped with a 60 ft boom using 15 gallons of water per acre (GPA). The second applications were made with a smaller Mudmaster ground rig borrowed from Glenn Co Tractor Co, Willows, CA.

The straw trial area received only one application of 4 lb ai/ac Superwham at 28 DAS applied by the grower. This treatment was considered appropriate preparation for the application of Straw Management treatments to be made following harvest, and that will be evaluated at the end of the 2001 season. In addition it provided an opportunity for comparison to the two applications made in the Main Experiment as well as to the grower's practice of one application at the later stage in the rest of his field.

Other applications. On 5/19 (two weeks after planting) 5 lb/ac of copper sulfate was applied by air for control of algae and tadpole shrimp. The insecticide Warrior was applied for control of rice water weevil and for an infestation of armyworms. One application of the fungicide Quadris was applied on 8/3 at the heading stage for control of blast.

Watergrass plant density data. The emergence of rice and watergrass seedlings through the floodwater during the first month was monitored using a five-gallon bucket with transparent plastic bottom. This quadrat of area 0.49 ft^2 was placed at 11 points within each plot along two north-south transects across the width of the plots giving a total sampled area of 1 m^2 per plot. The density of underwater watergrass seedlings was recorded at 13, 19, and 25 DAS by observing them in this way through the transparent plastic. Midseason watergrass plant densities visible above the rice canopy were estimated by counting the number of plants in a 1 m^2 quadrat, and then visually extrapolating to the whole plot area. Similar visual evaluations of herbicide trials based on subjective estimates of cover are standard practice. In this case, conversion to a density scale, instead of the more common "percent control" or 1 to 10 scales, was used in order to provide the population dynamics parameters necessary to connect to the seed bank densities and provide long term and continuous evaluation of treatment effects. These data were taken at 48, 51, 61, 69 and final plant density at 83 DAS. Plant survival values were calculated from the plant densities before and after treatment. Survival values, (or 100 - survival = %control or field efficacy) were used to evaluate the field performance of each treatment without reference to an untreated check. Comparison with greenhouse efficacy tests for resistance provides information on the role played by rice competition and water management in providing additional weed suppression. The density of watergrass panicles was also estimated in this way at 115 DAS. Samples of panicles were also taken in order to determine the number of seed per panicle.

Harvest. On 10/13/00 the grower's harvester was used to cut a strip down the middle of each plot of the Main Experiment. Harvested area was 18 ft x 300 ft = 5400 ft2 = 0.1240 ac. Rice height, lodging percentage, and moisture were recorded for each plot. Transgenic rice and rice treated with experimental herbicides was put into a dumpster and taken to a landfill. The Foliar Herbicide Screening trial and the Exploratory trial were harvested on 10/15/00.

Straw management treatments applied. On Oct 12, nine 320 ft x 48 ft plots of the Straw Management trial were laid out to accommodate 3 replications of 3 treatments each in an RCB design (Fig. 1), blocking against an observed watergrass density gradient which varied from about 2 plants/m2 on the north to about 6/m2 on the south end. It was noted that the weeds smallflower umbrellaplant (*Cyperus difformis*) and redstem (*Ammannia coccinea*) also reseeded in this area. The soil seed bank in each plot was sampled in a stratified random pattern of four 4" diameter soil cores per plot. Each core was divided into an upper 0 to 5 cm layer and lower 5 to 12.5 cm layer and bulked by layer and plot. The three straw treatments are (1) burn, (2) chop, and (3) chop and incorporate. Straw in all plots was first chopped on Oct 24. On Oct 25 the straw in plots receiving treatment 1 was burned after a water truck wet the straw on the borders to contain the fire. A good burn was accomplished (about 95% complete) even though it began to rain before it was out. The rain delayed incorporation of straw with a chisel implement in treatment 3 until Nov 8 (and prevented incorporation of straw in the main plots). These straw treatments and other essential fall work would not have been possible without an especially outstanding effort by the cooperating grower, Mr. Larry Maben.

Post-harvest work and seed bank sampling. Final rice plant and tiller density counts were recorded from the rice stubble in a 1 square foot quadrat placed four times in each plot of the Main Experiment and in each plot of the Exploratory trial. Plots of the main Experiment were sampled in a stratified random pattern of eight 4" diameter cores per plot. Each core was divided into an upper 0 to 5 cm layer and lower 5 to 12.5 cm layer and bulked by layer and plot. Rice straw in the entire 21.2 acres was chopped by the grower with a flail mower. Levees in the Exploratory trial were pushed down with a bulldozer. This area and another badly rutted area in the northwest corner were then chiseled. The field was flooded on 11/9/00.

Results and Discussion

a. Research

Post-harvest seed bank densities in fall 1999. In the Main Experiment, the density of new early watergrass seed recovered from the soil surface after harvest (Table 1) was 2 to 3 times the preplant seed density in the propanil and glufosinate treated plots and about 7 times in the molinate treated plots. That is, even though propanil and glufosinate achieved about 95% control in limiting the watergrass plant density to the 4 to 8 per square meter range, the watergrass population increased; this illustrates the high potential for reinfestation given the large amount of seed that even a few plants can produce. For this reason successful management of resistance must focus on the depletion of the soil seed bank. The high rice yield of the propanil treated plots indicated that the competition exerted by a watergrass density of about $4/m^2$ might not have significantly reduced yield, however. Taken together these results suggested that in order to effectively reduce reseeding and bring this resistant population under control, weed density must be kept lower than a level required merely to avoid significant yield loss. Further, doing this in the field will require greater than 95% control. Therefore it was decided to use two applications of herbicides in the year 2000 in order to eliminate potential survivors (resistant plants) and late-emerging watergrass capable of shedding new seed.

Recruitment (Table 1), defined here as the number of apparently viable seed produced per mature plant, was significantly less at the higher plant densities of the molinate treated plots than at the lower plant densities where the herbicide was more effective. This may result from density-dependent effects on seed production per plant of watergrass even though mature plant density in the molinate treated plots was roughly 10 times that in the propanil and glufosinate treated plots, new seed density was higher by only a factor of about 2 (Table 1). These data allow us to improve our estimates of seed recruitment based on final plant densities. With no previous data as guide, predicted seed rain was considerably overestimated in last year's report.

Post-harvest densities of old seed were 44% of the preplant densities and did not vary significantly by treatment (Table 1). This value is slightly less than the 58% measured in a previous study (Hair, 1996). Survival in the upper 2" layer of soil was significantly less (22%) than at the lower 2" to 4" depth (66%)(Data not shown). This difference not only implies that watergrass germination into the rice crop occurs mainly from the upper soil layers, but suggests that a strategy of depletion of the upper soil layer by germination, prevention of reseeding by effective herbicide use, followed by a no-till planting method that does not turn up buried old seed, could result in much reduced weed emergence. Just such a strategy was successfully implemented and demonstrated in the no-till plots of the exploratory trial conducted this year. Reseeding in this area was minimized by the grower's herbicide program in 1999 (thiobencarb 4 lb ai/ac at 2 lsr + propanil 6 lb ai/ac at tillering stage). One non-replicated fall sample of 4 cores from this area recovered a seed density of $430/m^2$, only 16% of which were new seed (Table 3).

Preplant seed bank assessment May 2000. The average density of apparently viable seed recovered from the soil samples taken May 2000 in the Main Experiment was only $460/m^2$ and did not vary significantly by treatment (Table 1). Survival over the winter and spring following a straw management treatment of burning and winter flooding was surprisingly low – only about 4%. In spite of the rather large density of new seed in the fall of 1999 discussed above, the preplant seed density in 2000 was only about 14% of the preplant density in 1999 at the beginning of the study. The cultural practices of burning straw and winter flooding were chosen in the first year of this project because other work has indicated that these operations would be most advantageous in preventing a build-up of watergrass seed (Hair, 1996, and Hair, et al, 1999). The 96% loss observed here appears to support those findings. Under current law however, growers can burn only rarely, and so after this year's harvest, straw was chopped in the herbicide trial before flooding for the winter. Rain prevented a planned incorporation operation that would have promoted straw decomposition. The mechanisms and details of straw management effects on watergrass seed bank dynamics will be studied further in a separate experiment (Straw Management trial) at this site (Figure 1) where burn, chop, and incorporated straw treatments, all winter flooded, will be compared. It is expected that predation from the soil surface and germination in early spring will be the main loss mechanisms, and that both will be enhanced by straw removal and by avoiding seed burial. If this is correct, then chopping and flooding without the seed burial that incorporation involves may become the preferred alternative to burning.

In early April before tillage had begun, an average of 12 watergrass seedlings per m^2 had emerged in the Main Experiment; no emergence was found in the new plots of the Straw Management trial or southwest Exploratory trial where reseeding had been largely prevented the previous year. This difference is consistent with the interpretation that germination from the surface can be a significant mechanism by which watergrass seed is lost form the soil seed bank. Also, the rain that interrupted seedbed preparation this spring (20 days before seeding) apparently enhanced watergrass seed losses through spring germination; in this second flush 29 seedlings per $m^{2\ were}$ counted in the tilled plots of the Main Experiment, 15 per m^2 in the tilled plots of the Exploratory trial and the Foliar Herbicide Screening trial, and 7 per m^2 in the non-tilled plots of the Exploratory trial. In general these results support the idea that tillage is a major determinant of watergrass population dynamics through its effect on seed depth, germination, and predation (Hair, 1996).

Emergence of rice and watergrass seedlings. Rice and watergrass emerged very slowly due to cool cloudy weather for 10 days after seeding. Rains 1 DAS and again at 8 DAS not only kept the sky cloudy but caused a loss of control in the no-till plots of the exploratory trial using pendimethalin (one of the few remaining herbicides to control resistance watergrass, which can only be used under dry-seeding conditions). The first rice leaf finally emerged 12 DAS when record high temperatures were recorded. In most plots of the Main Experiment the rice stand that then quickly established was rather thin, but acceptable (17 plants/ft^2 or 90 plants per m^2, 13 DAS). The Liberty-Link rice seed however germinated poorly (5 plants/ft^2) and had to be reseeded at 17 DAS. Given the low preplant seed densities in the Main Experiment discussed above, it was surprising to observe average watergrass seedling densities of 97 plants/m^2 at 19 DAS, only slightly less than was observed in 1999 (Table 2). There is no ready explanation why emergence was 3.5 % of the preplant seed density in 1999 and 21% in 2000. We can only speculate that perhaps moisture and temperature differences associated with the rain and the unplaned seedbed may have played a role; or perhaps there was a higher proportion of new seed this year than last (see discussion below).

In the Exploratory trial where presoaked seed was waterseeded, the rice stand at 13 DAS was 27 plants/ft^2 in the tilled plots and 12 plants/ft^2 in the non-tilled plots (data not shown). Although rice establishment was poor in these no-tilled plots (some of the broadcasted rice seed remained caught on the old rice stubble without reaching the ground) the strategy of taking advantage of an upper soil layer depleted of watergrass seed (probably helped considerably by the rain), little reseeding the previous year, burning of straw, and not turning up old buried seed, succeeded dramatically in preventing watergrass emergence. At 18 DAS watergrass seedling density in the tilled plots of the molinate (Ordram) treatment was 25 plants/m^2, but none was found in the non-tilled plots (Table 3). Although no herbicide was used in the non-tilled plots of the Exploratory trial, final watergrass density at 83 DAS was only 1 plant per 100 m^2. Analysis of the preplant soil samples confirm that the upper 2.5 cm layer must have had a seed density less than about 12/m^2 (Note 3/ in Table 3). Emergence in the tilled plots of this trial was about 4% contrasted with 21% in the main plots mentioned above. Since the seedbed preparation was similar in these two trials in the same year, this result suggests that the older seed in this trial may have caused the difference. If this is true even more emphasis should be placed on the prevention of reseeding. Also, it may help to explain why the density of weed emergence for other weeds and cropping systems has such notoriously poor correlation with preplant seed densities (Forcella, et al 1992, Hair, 996). We should learn more about this topic in this trial in 2001 but it should be examined in controlled experiments in the future.

Performance of herbicide use strategies in the Main Experiment. A mean of 97 plants/m^2 was established in all plots of the herbicide trial at 19 DAS with no significant difference by herbicide treatment strategy (Table 2). Mature watergrass density at 83 DAS indicated that survival of the molinate treatment in 2000 was 6.3% -- considerably lower than the 69% survival observed in the first year. This was probably due to deeper water depth and to the split application at a higher total rate in 2000. The resistance of this watergrass population is indicated by the failure of the first application of molinate to control the rapidly growing watergrass seedling density (50% survival, Table 2). Overall survival of the 2 applications of the foliar spray treatments were also roughly a factor of 10 lower than the one comparable application in 1999 (Table 2). All were significantly lower than the molinate treatment (Table 2).

It appeared that the first application of the combination treatment (Abolish/Regiment) was much more effective than the second (Clincher/Super Wham). Also, the first application of propanil in the rotational treatment was more effective than the second. In the glufosinate treatment the second application appeared to be more effective, but this may have been simply because the first was applied at a lower rate than intended, and because of the replanting of the Liberty-Link plots 17 DAS. All three treatments succeeded in limiting severely the input of new seed into the seed bank. In terms of overall percent control, we may say that the propanil treatment achieved about 99% control, the intensive tank mixed combination treatment about 99.9% and the glufosinate treatments about 99.99% control. These treatment means differ by roughly one order of magnitude between each. It will be very interesting to track these differences through the recruitment, winter seed survival, and next year's emergence phases to ascertain whether these levels of herbicidal control will make a difference in weed pressure in the following year. Even more interesting is the possibility that since selection pressure is intensified at these higher levels of control, we may still hope to observe different changes in the resistance patterns of seed extracted from the seed bank next year.

Herbicide performance and rice yields in the Exploratory trial. The molinate treatment in these plots was exactly the same as that of the main plots, but watergrass control was much better; watergrass survival (2%) was less than one third survival in the main plots (6%) (Tables 2 and 3). This may have been due to slightly deeper water, but also perhaps due to the older seed in this area (see discussion above on emergence). Final plant density of 0.3 plants/m^2 represents quite good control. Perhaps a third possible explanation is that two years of very good control with propanil in this area may have reduced the proportion of molinate resistant seed in the seed bank; that is, the population may have become more susceptible. The pendimethalin application was made to the soil surface before emergence of rice and watergrass. Heavy rains 4 and 5 days after treatment may have carried the herbicide below the very top layer of the soil, where germinating watergrass seed is, towards the deeper rice coleoptiles causing severe rice injury. The result was loss of control and a thin stand of rice. This herbicide may have a role in certain special situations in the future but methods must be found to reduce risk – perhaps a later timing in combination with another herbicide. Rice yields in all treatments were low and not different from that of an untreated check, which had 14 watergrass plants/m^2 (Table 3). Shorter rice in the molinate treated plots (data not shown) may indicate that the split application of a total of 5 lb ai/ac was injurious. That is, there may have been a yield penalty to achieve the increased control. The low yields of the no-till treatment may have been due to problems of rice establishment or fertilization. This is a reminder that any weed control practice, whether cultural or herbicidal, must be integrated into a workable overall system.

Herbicide performance in the Foliar Herbicide Screening trial. Results with the ten-herbicide treatments may be divided roughly into three groups: 1. Applications of thiobencarb and cyhalofop provided no control at all. These two treatments and the untreated check all had about 5 watergrass plants/m^2 and resulted in similar yields as the untreated check (Table 4). This was expected since greenhouse tests in 1999 showed resistance to thiobencarb and to fenoxaprop, an herbicide with the same mode of action as cyhalofop. 2. Treatments # 4, 5, 6, 7, and 10 (Table 4) involving propanil and combination with four other herbicides provided a medium level of control with values in the 79 to 91% range, and watergrass densities of about 1 plant/m^2. 3. The remaining two treatments of bispyribac-sodium alone and in combination with thiobencarb provided control of 95 and 100% respectively (0.2 and 0 plants/m^2). These results confirm what was discussed in last year's report regarding bispyribac; although resistance was indicated by the greenhouse tests, efficacy in the field remains high (Fischer et al, 2000). These results also confirm that the synergism between thiobencarb and bispyribac that has been seen in other trials appears to be working on this resistant population as well. The use of synergistic combinations is a not easy to find key tool for delaying the development of resistance to a given herbicide.

Rice grain Yields. The continuous molinate treatment in the Main Experiment yielded significantly less than the other foliar treatments, presumably because of the poorest watergrass control, which left an average of 6 plants/m^2 (Table 2). Final rice height in the molinate plots was significantly lower, however, indicating that perhaps rice injury may have played a role, given the dual application and overall higher than normal rate used with this herbicide. The propanil treated plots (two sequential applications) had the highest yield (~1 watergrass plants/m^2) even though the other foliar treatments had better watergrass control (0.1 and 0.01 plants/m^2). The lower yields in the glufosinate plots may be attributable to the experimental nature of the Liberty-Link rice seed used in this experiment.

b. Demonstration and Extension

Grower field tour and grower winter meetings. A tour and discussion of these issues was held at the field site on August 9, 2000. The meeting was announced in a newsletter sent to everyone on the Farm Advisor's mailing list in Glenn, Colusa and Yolo counties. We were pleased at the turnout; 20 growers and 12 PCAs or other interested people attended. The turnout and the discussions that followed appear to indicate a serious concern among growers about watergrass resistance. Also, these results are being reported and discussed by the authors at four different UC Cooperative Extension sponsored winter grower meetings in February 2001.

Summary and Conclusions

In this second year of the project the herbicide trial was planted, treated and evaluated, an expanded site area was prepared for the application of straw management treatments after harvest, part of a demonstration area was utilized to test tank mixed combinations of herbicides on this resistant watergrass population, and a separate small trial was installed and conducted that explored alternative chemistries and the alternative planting methods of drill and no-till seeding. The results of seed bank sampling produced several important results: 1. Higher than expected recruitment of new seed from rather low plant densities indicated the necessity of better herbicidal control to prevent reseeding. 2. The upper layers of soil were depleted of old seed significantly more than the lower levels. 3. The seed bank was reduced over winter and spring by 95% after burning and flooding straw treatments. 4. Emergence of watergrass seedlings was 30% of the preplant seed bank in 2000 contrasted with 3.5% in 1999. Tracking watergrass plant densities throughout the season provided a means for evaluating the effects of two herbicide applications in each of the four treatments. These data indicated the failure of molinate to provide acceptable control on this resistant population even with split applications of a higher total rate and with improved water management. The two applications of all three foliar treatments achieved levels of control judged probably adequate to deplete the seed bank by limiting the recruitment of new seed, and possibly intensifying selection pressure enough to alter the patterns of resistance in the seed bank. As a practical matter this project will do much to clarify which herbicide strategies and cultural practices are most advantageous for bringing resistant watergrass populations under control. At the same time, from a more fundamental perspective, this project will advance the integration of population and seed bank dynamics with herbicide strategies and evolution of resistance concepts producing more comprehensive models for long term planning.

The experiment has also demonstrated its potential as a much-needed tool to enhance farmer's awareness of the magnitude of the herbicide resistant problem that is crippling weed control in California rice. At the same time, through the field tour and subsequent presentations at growers' meetings, valuable concepts and techniques from the "real world" are being forwarded to growers. The high turnout to these events are testimony of farmers' need for solutions and of the relevance and potential of this applied research and demonstration project.

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APPENDICES

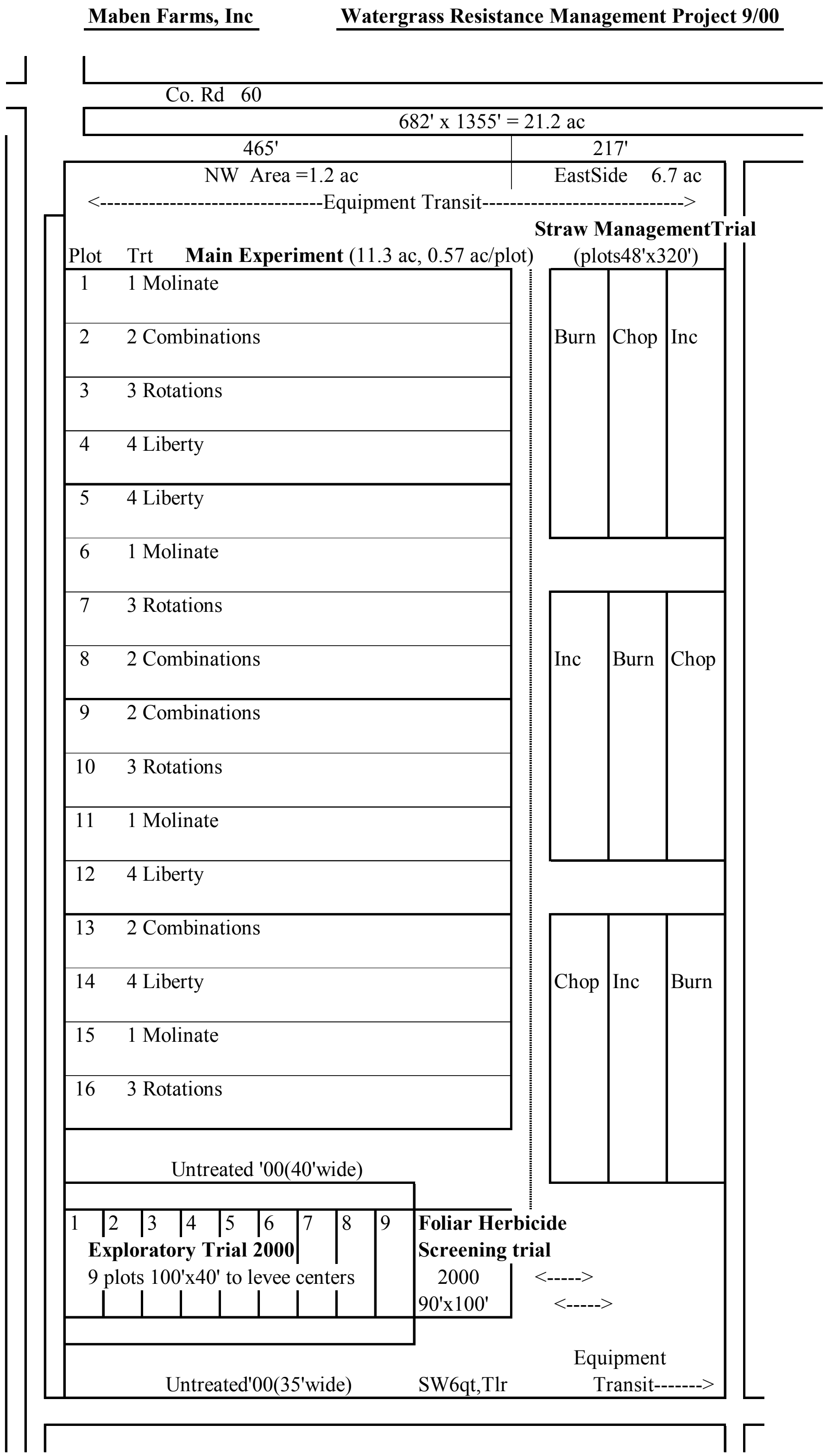

Figure 1. Field layout of experimental treatments.

Table 1. Early watergrass (*Echinochloa oryzoides*) densities and survival and rice grain yield under different herbicide strategies in the main plots during 1999. Means of 4 replications.

Herbicide Strategy	Seed Preplant	Watergrass Density				Plant Survival	Panicle Density	Rice Yield	Watergrass Seed after harvest		
									New	Recruit	Old
	(#/m2)	Days after seeding				(%)	(#/m2)	(1b/ac)	(#/m2)	(#/plant)	(#/m2)
DAS:		14-21	37	42	84		123	154			
			(#/m2)								
1. Molinate	3300	108	143 a	76 a	63 a	69 a	508 a	4800 c	22,050	380	1360
2. Propanil	3250	99	20 b	6 a	4 b	5 b	68 b	9990 a	5,850	1400	1230
3. Glufosinate	3450	122	19 b	0.1 b	8 b	6 b	97 b	8840 b	9,960	1320	1250
4. Glufosinate	3510	161	6 b	0.1 b	7 b	5 b	103 b	9280 b	8,580	1440	1630
Grand Mean	3380	122	-	-	-	-	-	8230	11,600	-	1370
Prob > F	0.89	0.09	0.001	0.002	0.0002	0.0001	<.0001	<.0001	0.50	0.24	0.74
CV (%)	17	26	64	*	*	*	25	2.7	*	*	41

Notes:

1/ Seedling emergence as percent of preplant seedbank was 122/3380 = 3.6% uniformly in all plots.

2/ Molinate applied 9 DAS, 1 lsr, 4 lb ai/a. All foliar applications 27 DAS, 2 tiller rice, drained field, ground rig. Propanil 4 lb ai/ac Super Wham, 1.25% COC. Glufosinate 0.36 lb ai/ac Liberty, + 3lb/ac ammonium sulfate.

3/ Plant survival calculated from densities at 84 DAS relative to seedling density at 14-21 DAS.

4/ Recruitment = ratio of new seed density to plant density at 84 DAS.

5/ Seed survival over summer was 1370/ 3380 = 41%. (22% in upper 2" soil layer, 66% in lower; data not shown)

6/ Means in same column followed by different letter significantly different. Means separation by protected LSD test at 0.001 significance level in all columns except plant density at 84 DAS, 0.05 level, and rice yield, 0.01 level. Molinate values for new seed (p=0.06) and recruitment (p=0.02) significantly different by orthogonal contrast.

7/ *Asterisk indicates variables transformed (either Log or square root) for analysis to obtain homogeneous variance and/or normal error distribution. CV not applicable.

Table 2. Early watergrass (*Echinochloa oryzoides*) densities and survival and rice grain yield under different herbicide strategies in the main plots during 2000. Means of 4 replications.

Herbicide Strategy	Seed Preplant (#/m2)	Watergrass Density Days after seeding				Plant Survival (%)			Panicle Density (#/m2)	Rice Yield (1b/ac)
						1st app	2nd app	Both apps		
DAS:		19	25	51-61	83				115	160
			(#/m2)							
1. Continuous molinate	480	105	53 a	4	6 a	50 a	11 a	6 a	23 a	7860 c
2. Intensive Strategy	360	89	105 b	0.14	0.1 c	0.2 b	41 a	0.1 c	0.16 c	8910 ab
3. Herbicide MOA Rotatic	500	92	95 b	3	0.8 b	4 b	52 a	0.9 b	4 b	9260 a
4. Continuous glufosinate	500	104	115 b	5	0.01 c	6 b	0.5 b	0.01 c	0.05 d	8310 bc
Grand Mean	460	97	-	-	-	-	-		-	8580
Prob > F	0.76	0.58	0.004	0.18	0.006	0.02	0.02		<.0001	0.008
CV (%)	47	20	19	*	*	*	*		*	5.3

1/ Winter and spring survival under a burn and flood straw management practice was 460/12,970 = 3.5%.
2/ Seedling emergence as percent of preplant seedbank was 97/460 = 21% uniformly in all plots.
3/ Molinate applied 12 DAS, 1 lsr, 3 lb ai/ac followed by (fb) 2 lb ai/ac at 20 DAS 2.5 lsr. 1st foliar app by ground at 28 DAS, 4 lsr, 2nd at 54 DAS. Intensive Strategy: Abolish 4 lb/ac + Regiment 15 g ai/ac fb Cllincher 210 g ai/ha + SuperWham 6 qt/ac.
Herbicide Mode of Action (MOA) Rotation: SuperWham 4 qt/ac fb Superwham 6 qt/ac. Continuous glufosinate (Liberty) 350 g ai/ha fb Liberty 500 ga ai/ha.
4/ Plant survival of 2 apps of molinate calculated from densities at 19 to 25 and 25 to 51-61 DAS.
Plant survival of foliar treatments calculated from densities at 25 to 51-61 and 51-61 to 83 DAS for 2 applications.
5/ Means in same column followed by different letter significantly different. Means separation by protected LSD test at 0.05 significance level in all columns except plant density at 25 DAS, 0.01 level.

Table 3. Early watergrass (*Echinochloa oryzoides*) densities and survival and rice yield under different herbicide strategies in the exploratory trial in 2000. Means of 3 replications.

Treatment	Plant Density	Seed Preplant		Plant Density			Plant Survival	Panicle Density	Rice Yield
		Top	Total						
DAS:	-11	-1	-1	18	55	83	–	115	-
	(#/m2)						(%)	(#/m2)	(lb/ac)
1. Molinate	14	214	619	25	1	0.3	2	0.6	6320
2. No-Till	7	0	393	0	0.1	0.1	-	0.4	5830
3. Pendimethalin	16	179	762	29	22	7	36	19	5590
Untreated Check	-	-	-	30	14	14	50	41	6250
Prob > ChiSq	NS	0.05	NS	0.06	0.02	0.05	NS	0.07	NS

1/ In 1999 trial area treated with Bolero 4 lb ai/ac fb SuperWham 6 lb ai/ac
Fall 1999 non-replicated soil sample result: ~430 seed/m2; ~84% old seed
2/ Plant density 11 days before seeding indicated spring germination loss due to rain at -20 DAS.
Treatments 1, 2 chiseled before and after rain. No-till plots received Roundup (2%) -5 DAS.
3/ No seed found in top 2.5 cm layer of no-till treatment; sample area 0.084 m2; density < 12/m2 (if 1 plant was found in this area).
4/ Emergence in tilled plots of treatment 1 and 2 was 3.9%
5/ Plant survival derived from plant densities at 83 and 18 DAS.
6/ Untreated check not replicated. Not used in analysis.
7/ Probability > chi squared value from non-parametric Wilcoxon test. ANOVA not applicable.

Table 4. Weed control, early watergrass (*Echinochloa oryzoides*) density, and rice yield in the auxiliary herbicide trial during 2000. Means of 4 reps.

Treatment 2/	Rate	Weed Control CYPDI 1/ 10 DAT 3/	Weed Control ECHOR 20 DAT	Weed Control ECHOR 60 DAT	Watergrass Density 50 DAT	Rice Yield 4/
	(g ai/Ha)		(%)		(#/m2)	(lbs/A)
1. Untreated 5/	–	11	24	23	4.7	7650
2. thiobencarb (EC)	4480	0	0	0	5.7	7180
3. cyhalofop + COC	280 + 1.25% v/v	0	36	0	4.9	7760
4. propanil (SC) + CC	6720 + 1.25% v/v	85	69	89	0.9	8730
5. Duet (propanil + bensulfuron) + COC	(6720 + 85) + 1.25% v/v	95	70	86	1.5	8270
6. propanil SC + thiobencarb (EC)	4480 + 4480	75	63	79	1.6	8585
7. propanil SC + cyhalofop + COC	4480 +210 + 1.25% v/v	36	94	91	0.7	8150
8. bispyribac-sodium + Silwet	37 + 0.125% v/v	65	94	95	0.2	8320
9. bispyribac-sodium + thiobencarb (EC)	37 + 4480	0	98	100	0	8050
10. propanil SC + bispyribac-sodium + Silwet	4480 + 30 + 0.125% v/v	30	83	89	1.4	7790
LSD (0.05)					1.9	720

1/ ECHOR = early watergrass; CYPDI =smallflower umbrellasedge
2/ Treatments applied at 2-3 tiller stage of rice.
3/ DAT = days after treatment COC = crop oil concentrate
4/ Yield adjusted to 14% moisture content
5/ Untreated values are percent cover by weed species